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End of Result Set

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File: DWPI

L7: Entry 1 of 1

DERWENT-ACC-NO: 1972-03539T
DERWENT-WEEK: 197203

TITLE: Ovular and seminal cell extracts - having a haploid chromosome set for therapeutic and diagnostic use

PATENT-ASSIGNEE:

ASSIGNEE	CODE
THEURER K	THE I

PRIORITY-DATA: 1970DE-2032988 (July 3, 1970)

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
DE 2032988 A		N/A	000	N/A

INT-CL (IPC): A61K 17/00

ABSTRACTED-PUB-NO: DE 2032988A
BASIC-ABSTRACT:

Prepn. and use in therapy and diagnostic use and ovular cell extracts having a haploid chromosome set from corpuscular and high molecular fractions (nucleic) acids, proteins, lipides and polysaccharides) and their degradation products, for aiding cell-proliferation and treating sterility and fertility.
Antibody-sera against fractions with X- or Y-chromosomes can be used for sex-determination. Cells from fish are esp. suitable as starting materials. In example, for an antibody-serum against bovine Y-chromosomes cell-nuclei are obtained by grinding oval cells from trout-roe in liquid N2, centrifuging in a saccharose-gradient, freeze-drying and decomposing chemically. Antibody serum from rabbits immunised with the oval product is incubated with bull-sperm. The non-agglutinated sperm-cells are isolated and the nuclei from the spermatozoa with Y-chromosomes used for the active immunisation of rabbits or sheep.

TITLE-TERMS: OVUM SEMEN CELL EXTRACT HAPLOID CHROMOSOME SET THERAPEUTIC DIAGNOSE

DERWENT-CLASS: B04

CPI-CODES: B04-B02D; B04-B04A; B04-B04C; B12-G01; B12-G04; B12-J01; B12-K04; B12-L05;

CHEMICAL-CODES:

Chemical Indexing M1 *01*
Fragmentation Code
V622 V624 V795 V751 V752 V753 V754 V600 V611 N160
P610 P620 M720 P710 P831 P832 M781 P930 Q252 R000
M423 M902

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L12: Entry 2 of 3 File: USPT Jun 6, 2000

DOCUMENT-IDENTIFIER: US 6071689 A
TITLE: System for improving yield of sexed embryos in mammals

ABPL:
Improved insemination systems particularly adapted to use for sex-selected sperm sorting include systems which achieve superovulation and then multiple embryo production with sexed embryos. These systems combine with other techniques, including techniques for enhanced sheath fluid and other strategies which minimize stress on the sperm cells, and, potentially, a 2.9 percent sodium citrate sheath solution for bovine species and a hepes bovine gamete media for equine species. Improved collection systems and techniques for the process are described so that commercial application of sperms samples as well as the resulting animals may be achieved.

BSPR:
For ages it has been desired to select the sex of specific offspring. Beyond obvious psychological aspects, the actual sex selection of mammalian offspring has significant economic consequences when one considers its application to food producing animals such as cattle as well as celebrated trophy animals such as horses and the like. This great desire has resulted in a significant variety of efforts to achieve sex-selected offspring. Probably the effort which has appeared most likely to achieve the desired results has been efforts at sorting and selecting between X and Y sperm prior to insemination.

DEPR:
Another aspect which may interplay in the various factors of the present invention is that of utilizing low dose amounts of sperm for artificial insemination or the like. Additional background on the aspect of sexed, artificial insemination may be found in "Prospects for Sorting Mammalian Sperm" by Rupert P. Amman and George E. Seidel, Jr., Colorado Associated University Press (1982) hereby incorporated by reference. As mentioned, natural insemination involves numbers of sperm on the order of billions of sperm. Typical artificial insemination is presently conducted with millions of sperm for bovine species and hundreds of millions of sperm for equine species. By the term "low dose" it is meant that the dosage of sperm utilized in the insemination event are less than one-half or preferably even less than about 10% of the typical number of sperm provided in a typical artificial insemination event. Thus, the term "low dose" is to be viewed in the context of the typical artificial insemination dosage or also as an absolute number. For bovine sperm where currently 1 to 10 million sperm are provided, a low dose process may be considered an absolute number of about 500,000 sperm or perhaps as low as 300,000 sperm or lower. In fact, through utilization of the techniques of the present invention, artificial insemination with good percentages of success has been shown with levels of insemination of sperm at 100,000 and 250,000 sperm (41% and 50%, respectively pregnancy rates). As shown in the article "Uterine Horn Insemination of Heifers With Very Low Numbers of Non-frozen and Sexed Spermatozoa" as published in 48 Theriogenology 1255 (1997) hereby incorporated by reference. Since sperm cells appear to display a sensitivity to dilution, these results may display particular interdependence on the utilization of low dose sperm samples with regards to various techniques of the present invention. The absolute numbers may be species dependent, for equine species, merely less than about ten, five, or even one million sperm may be considered a low dose process.

DEPR:
As examples, the following experiments have been conducted. While not all use every aspect of the inventions described here, they do show the performance enhancements possible through differing aspects of the invention. Further, a summary of some experiments is contained in the article "Uterine Horn Insemination of Heifers With Very Low Numbers of Non-frozen and Sexed Spermatozoa" as referenced earlier. This article summarizes some of the data showing the efficacy of the present invention. As to the experiments, one has been conducted with sexed, unfrozen sperm cells with high success as follows:

DEPR:
Angus heifers, 13-14 mo of age and in moderate body condition, were synchronized with 25 mg of prostaglandin F-2 alpha at 12-day intervals and inseminated 6-26 h after observed standing estrus. Freshly collected semen from three 14-26 mo old bulls was incubated in 38 .mu.M Hoechst 33342 at 75.times.10.sup.6 sperm/ml in a TALP medium for 1 h at 34.degree. C. Sperm were sorted by sex chromosomes on the basis of epiflourescence from laser excitation at 351 and 364 nm at 150 mW using a MoFlo.RTM. flow cytometer/cell sorter operating at 50 psi and using 2.9% Na citrate as sheath fluid. X chromosome-bearing sperm (.about.90% purity as verified by resorting sonicated sperm aliquots) were collected at .about.500 live sperm/sec into 2-ml Eppendorf tubes containing 100 .mu.l Cornell Universal Extender (CUE) with 20% egg yolk. Collected sperm were centrifuged at 600.times.g for 10 min and resuspended to 1.63.times.10.sup.6 live sperm/ml in CUE. For a liquid semen unsexed control; Hoechst 33342-stained sperm were diluted with sheath fluid to 9.times.10.sup.5 sperm/ml and centrifuged and resuspended to 1.63.times.10.sup.6 progressively motile sperm/ml in CUE. Sexed semen and liquid control semen were cooled to 5.degree. C. over 75 min and loaded into 0.25-ml straws (184 ul/straw). Straws were transported at 3 to 5.degree. C. in a temperature-controlled beverage cooler 240 km for insemination 5 to 9 h after sorting. Sexed semen and liquid control semen were inseminated using side-opening blue sheaths (IMV), one half of each straw into each uterine horn (3.times.10.sup.5 live sperm/heifer). As a standard control, semen from the same bulls had been frozen in 0.5-cc straws by standard procedures (mean 15.6.times.10.sup.6 motile sperm/dose post-thaw), thawed at 35.degree. C. for 30 sec, and inseminated into the uterine body. Treatments were balanced over the 3 bulls and 2 inseminators in a ratio of 3:2:2 inseminations for the sexed semen and two controls. Pregnancy was determined ultrasonically 31-34 days after insemination and confirmed 64-67 days later when fetuses also were sexed (blindly). Data are presented in the table.

DEPR:
The objective was to determine pregnancy rates when heifers are inseminated with extremely low numbers of frozen sperm under ideal field conditions. Semen from three Holstein bulls of above average fertility was extended in homogenized milk, 7% glycerol (CSS) extender plus 5% homologous seminal plasma to 2.times.10.sup.5, 5.times.10.sup.5 or 10.times.10.sup.6 (control) total sperm per 0.25 ml French straw and frozen in moving liquid nitrogen vapor. Semen was thawed in 37.degree. C. water for 20 sec. Holstein heifers 13-15 mo of age weighing 350-450 kg were injected with 25 mg prostaglandin F-2-alpha (Lutalyse.RTM.) twice at a 12-day interval and inseminated with an embryo transfer straw gun and side-opening sheath, half of the semen deep into each uterine horn 12 or 24 h after detection of estrus. The experiment was done in five replicates over 5 months, and balanced over two insemination technicians. Ambient temperature at breeding was frequently -10 to -20.degree. C., so care was taken to keep insemination equipment warm. Pregnancy was determined by detection of a viable fetus using ultrasound 40-44 days post-estrus and confirmed 55-62 days post-estrus; 4 of 202 conceptuses were lost between these times. Day 55-62 pregnancy rates were 55/103 (53%), 71/101, (70%), and 72/102 (71%) for 2.times.10.sup.5, 5.times.10.sup.5 and 10.times.10.sup.6 total sperm/inseminate ($P<0.1$). Pregnancy rates were different ($P<0.05$) among bulls (59, 62, and 74%), but not between technicians (64 and 65%) or inseminations times post-estrus (65% for 12 h and 64% for 24 h, N=153 at each time). With the methods described, pregnancy rates in heifers were similar with

5.times.10.sup.5 and 10.times.10.sup.6 total sperm per inseminate.

DEPL:
Although the pregnancy rate with sexed semen was only 80% of controls, this difference was not statistically significant (>0.1). One pregnancy was lost by 64-67 d in each of the sexed and frozen control groups; 18 of 19 fetuses (95%) were female in the sexed group, and 20 of 30 (67%) were female in the control groups. The liquid semen control yielded a virtually identical pregnancy rate to the frozen semen control containing over 50 times more motile sperm (over 120 times more total sperm), demonstrating the efficacy of low-dose insemination into the uterine horns. We have altered the sex ratio in cattle significantly using flow cytometer technology and artificial insemination.

DETL:

Treatment	No. Heifers bred	No. Pregnant d31-34	No. Pregnant d64-67	No female fetuses
Sexed semen	45	20 (44%)	19 (42%)	18 (95%).sup.a
Liquid control	28	15 (54%)	15 (54%)	8 (53%).sup.b
Frozen control	29	16 (55%)	15 (52%)	12 (80%).sup.c

.sup.a,b Sex ratios of values with different superscripts differ (P < 0.02).

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L12: Entry 1 of 3 File: USPT Nov 21, 2000

DOCUMENT-IDENTIFIER: US 6149867 A
TITLE: Sheath fluids and collection systems for sex-specific cytometer sorting of sperm

ABPL:
Improved flow cytometer system particularly adapted to use for sex-selected sperm sorting include enhanced sheath fluid and other strategies which minimize stress on the sperm cells, including a 2.9 percent sodium citrate sheath solution for bovine species and a hepes bovine gamete media for equine species. Improved collection systems and techniques for the process are described so that commercial applications of sperms samples as well as the resulting animals may be achieved.

BSPR:
For ages it has been desired to select the sex of specific offspring. Beyond obvious psychological aspects, the actual sex selection of mammalian offspring has significant economic consequences when one considers its application to food producing animals such as cattle as well as celebrated trophy animals such as horses and the like. This great desire has resulted in a significant variety of efforts to achieve sex-selected offspring. Probably the effort which has appeared most likely to achieve the desired results has been efforts at sorting and selecting between X and Y sperm prior to insemination.

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DETL:

No female	Treatment	No. Heifers bred	No. Pregnant d31-34	No. Pregnant d64-67	No. Pregnant fetuses
	Sexed semen	45	20 (44%)	19 (42%)	18 (95%).sup.a
	Liquid control	28	15 (54%	15 (54%)	8 (53%).sup.b
	Frozen control	29	16 (55%)	15 (52%)	12 (80%).sup.c

.sup.a,b Sex ratios of values with different superscripts differ (P < 0.02).

ORPL:
"Insemination Of Heifers With Very Low Numbers Of Frozen Spermatozoa." G.E. Seidel, Jr., C.H. Allen, Z. Brink, M.D. Holland, and M.B. Cattell, Colorado State University, Fort Collins, Atlantic Breeders Cooperative, Lancaster, PA, DUO Dairy, Loveland, CO, Jul. 1996.

WEST

End of Result Set

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L14: Entry 3 of 3 File: USPT Dec 17, 1985

DOCUMENT-IDENTIFIER: US 4559309 A
TITLE: Flow cytometry-fluorescence measurements for characterizing sperm

BSPR:
To quantitate RNA content and DNA content/chromatin condensation a fresh semen sample (1-6 hours post emission) or frozen sample (allowing samples to be accumulated and sent to a laboratory) is treated with a detergent solution, stained with acridine orange (AO) and measured by flow cytometry (FCM); approximately ten minutes are required to measure 5,000 cells per sample and analyze the data with computer assistance. The following can be learned from a single measurement: (a) the percentage of each cell type in semen inluding, (i) mature sperm, (ii) immature sperm precursor cells representing all stages of development from spermatogonia to mature sperm, (iii) somatic cells, e.g. leukocytes; (b) normality/abnormality of sperm nuclear chromatin condensation. These measurements can be correlated with cell types in testis biopsies identified by two parameter FCM measurements (RNA vs. DNA) using acridine orange as the fluorescent probe and measuring simultaneously the red and the green fluorescent values of each cell and storing in computer memory the integrated values for each cell.

DEPR:
To quantitate RNA content and DNA content/chromatin condensation for each of many possible cell types and differentiation levels of the cells present in human semen, a fresh semen sample (1-6 hours post emission) or frozen sample (allowing samples to be accumulated and sent to a laboratory) was treated with a detergent solution, stained with acridine orange (AO) and measured by flow cytometry (FCM); approximately ten minutes are required to measure 5,000 cells per sample and analyze the data with computer assistance. The following can be learned from a single measurement: (a) the percentage of each cell type in semen including, (i) mature sperm, (ii) immature sperm precursor cells representing all stages of development from spermatogonia to mature sperm, (iii) somatic cells, e.g. leukocytes; (b) normality/abnormality of sperm nuclear chromatin condensation. These measurements were correlated with cell types in testis biopsies identified by two parameter FCM measurements (RNA vs. DNA) using acridine orange as the fluorescent probe being available for AO intercalation and any increase in red/red pulse green fluorescence is due to AO interaction with single strand DNA.

WEST

Generate Collection

L14: Entry 2 of 3 File: USPT May 7, 1996

DOCUMENT-IDENTIFIER: US 5514537 A
TITLE: Process and apparatus for sorting spermatozoa

BSPR:
Certain dyes such as ethidium bromide and acridine orange have been used to evaluate DNA content of spermatozoa. Fluorescence intensity of the dye is proportional to the amount of dye absorbed. B. L. Gledhill et al., "Identifying X- and Y-Chromosome-Bearing Sperm by DNA Content: Retrospective Perspectives and Prospective Opinions," in R. P. Amann et al. (eds.) Prospects for Sexing Mammalian Sperm, pp. 177-91 (1982), discloses the use of 4',6-diamidino-2-phenylindole (DAPI) staining, and epi-illumination with orienting-flow cytometry, to show that the mean DNA content of bovine spermatozoa from frozen semen exhibited two distinct, but overlapping peaks of fluorescence. These peaks were nearly equal in size, and were separated by a 3.9% difference in intensity.